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Immune Response to *Trichomonas vaginalis*
II. Genetic Linkage of Immune Response Gene(s) Regulating IgE Antibody Response to *Trichomonas vaginalis* in Mice

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Introduction

It is generally thought that protozoan infections rarely induce immediate hypersensitivity (including IgE antibody production), although protozoan antigens have been reported to be effective for inducing delayed-type hypersensitivity (Sadun, 1972). In the experimental condition, however, the protozoan antigens, eg. *Entamoeba histolytica* (Usawattanakul *et al.*, in press), *Toxoplasma gondii* (Watanabe and Kobayashi, in preparation) and *Trichomonas vaginalis* (Yano *et al.*, 1982), have been reported to have the antigenicity to elicit IgE antibody in mice.

Meanwhile, series of H-2-linked immune response genes controlling the responses to various antigens, eg. synthetic polymers (McDevitt, 1968; Benacerraf and McDevitt, 1972), natural antigens (Vaz and Levine, 1970; Vaz *et al.*, 1971), H-Y antigens (Gasser and Silvers, 1971), etc. have been discovered. In this paper, we examined the genetic regulation of the IgE antibody response to *T. vaginalis* in mice. The results indicate that the IgE antibody response to the *T. vaginalis*-soluble extract is genetically regulated. At least two immune response genes are involved in controlling the IgE antibody response to *T. vaginalis* antigen; one localizes in the I-A and/or I-B subregion of the H-2 complex, the other in a not yet identified chromosome. We tried to map the non-H-2-linked immune response gene to immunoglobulin heavy chain constant region-linked locus (IgCH-linked locus), because McKenzie (1975) and Taylor *et al.* (1975) have shown that the antibody response to an alloantigen H-2.32 or chicken erythrocytes is subject to influence by IgCH-linked locus. The data of the experiments using allotype congenic mice show that the non-H-2 linked Ir-*T. vaginalis* is not linked to the IgCH-linked locus.

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Materials and Methods

Animals: C57BL/6CrSlc(B6), A/J, BALB/c CrSlc, B10.D2/nSn Slc and B10.A/SgSn Slc were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). BAB-14 and CB-20 mice were produced in our animal colony from stocks kindly provided by Drs. J. Eaton and B. Subbarao (The Institute for Cancer Research, Philadelphia, Pa. U.S.A.). BALB.B and BC-8 mice were

kindly supplied by Drs. H. Yamamoto (Kouchi Medical School, Kouchi, Japan) and S. Migita (Cancer Research Institute Kanazawa University, Kanazawa, Japan). Breeding pairs of both A.TH and A.TL were kind gifts of Dr. U. Yamashita (Kyushu Sangyo Medical School, Fukuoka, Japan). Mice of both sexes were used between 6 and 18 wk of age in this study. Recipient rats for passive cutaneous anaphylaxis (PCA) were 8–24 weeks old male Sprague-Dawley (Shizuoka Laboratory Animal Center).

Antigen: T. vaginalis lines (ATCC 3001 and PCA 110108) were generous gifts of Dr. M. Nishida (Fujisawa Pharm. Co., Osaka, Japan). The organisms were maintained in Trichomonas medium (Nissui Co., Tokyo, Japan) supplemented with 10% horse serum. The procedure for preparation of the soluble extract of *T. vaginalis* has been described previously (Yano *et al.*, 1982). Briefly, the cultured *T. vaginalis* was washed 5 times with 0.005 M PBS at 3,000 rpm for 15 min. After washing, the protozoa were sonicated with a Sonicator (Tominaga Industry, Co., Tokyo, Japan) for 60 seconds on ice and the sonicated material was ultracentrifuged at 10,000 rpm for 60 min. The supernatant was dialysed in PBS for 3 days. After dialysis, the material was again ultracentrifuged and sterilized by passing through millipore membrane (Millipore Co., Bedford, Massachusetts, U.S.A.). The protein concentration was measured by the method of Folin and Ciocalteu (1927), then the soluble antigen was adjusted to 5 mg/ml concentration and stocked at 4 C until use.

Immunization of Mice: As already described (Yano *et al.*, 1982), groups of 5–10 mice were immunized by an intraperitoneal injection of 30 μg of *T. vaginalis* antigen mixed with 2 mg $Al(OH)_3$, followed by a second injection of 30 μg of the antigen without adjuvant 14 days later. Animals were bled from the retroorbital plexus at frequent intervals, and the sera were subjected for passive cutaneous anaphylaxis (PCA) immediately after separation.

Passive Cutaneous Anaphylaxis (PCA): PCA was carried out as previously described (Ovary *et al.*, 1975). Briefly, 0.1 ml of serum dilutions were injected intradermally into the shaved backs of normal rats in duplicate. After 48 h, rats were challenged with an intravenous injection of 1 mg of soluble *T. vaginalis* antigen in 1 ml of 0.5% Evans blue dye solution. PCA titers were expressed as reciprocals of the maximum dilutions of antiserum that gave definite blueing more than 5 mm in diameter, read on the reflected skin.

Passive Hemagglutination Test: Passive hemagglutination (HA) was carried out by the method of Stavitsky and Arquilla (1955). Sheep red blood cells were coupled with *T. vaginalis* antigen by chromium chloride, as described by Gold and Fudenberg (1967).

Mercaptoethanol Treatment of the Sera: In order to identify IgM antibody in passive hemagglutination, the sera were treated for 2 hours at room temperature with 0.2 M 2-mercaptoethanol (2-ME) in PBS, pH 7.8 (Onoue *et al.*, 1967). Treatment with 2-ME drastically abolished passive hemagglutination (less than 1:8), these negative results are not shown in the tables.

Results

H-2 Linked Immune Response Gene Regulating IgE Antibody Response to T vaginalis

Series of Ir gene controlling the responses to various antigens have been found in the I subregion of H-2 in mice. In order to examine a possibility of the presence of Ir gene regulating IgE antibody response to *T. vaginalis*, we utilized various H-2 congenic mice. In preliminary experiments,

Table 1 The I-A and/or I-B subregion gene controls IgE antibody response to *Trichomonas vaginalis* antigen

Strain	No. of animals	H-2 complex								PCA titer[1]		HA titer[1]	
		K	I					S	D	mean[2]	range	mean[2]	range
			A	B	J	E	C						
C57BL/6	5	b	b	b	b	b	b	b	b	97.0	(32- 256)	222.9	(128- 512)
B10.A	5	k	k	k	k	k	d	d	d	111.4	(64- 256)*	337.8	(256- 512)
B10.S	5	s	s	s	s	s	s	s	s	<2.0	(0- 4)	168.9	(64- 256)
B10.S(9R)	5	s	s	?	k	k	d	d	d	3.0	(2- 16)†	147.0	(2- 512)
A.TL	6	s	k	k	k	k	k	k	k	362.0	(128-1,024)†	576.0	(256-1,024)
A.TH	5	s	s	s	s	s	s	s	d	97.0	(64- 128)†	407.3	(259- 512)
A/J	7	k	k	k	k	k	d	d	d	512.0	(256-1,024)*	337.8	(128-1,024)

1: Antisera were collected and separated on day 7 after secondary injection of 30 μg of *Trichomonas vaginalis* antigen.

2: Reciprocal dilution of antiserum, as calculated from geometric mean of either $\log_2$ PCA or HA titers.

*: significance between B10.A and A/J responses is $P<0.01$.

†: significance between A.TL and A.TH responses is $P<0.05$.

the maximum response of the IgE antibody was observed on day 7 of the secondary response. The HA response peaked at 10 to 14 days after the primary immunization, and the highest HA titer was obtained on day 7 to 10 of the secondary response. As shown in Table 1, C57BL/6 mice could produce both IgE and IgM antibodies to *T. vaginalis*. On the other hand, B10.S mice could produce IgM antibody (1:168.9), while this strain failed to raise the IgE antibody (0, 0, 1:2, 1:2, 1:4). These data indicate that the IgE antibody response to *T. vaginalis* is regulated by the H-2 linked Ir gene (Ir-*Tr. v.*). In order to map the Ir-*Tr. v.* into the I subregion, B10.S(9R) mice (recombinant between B10.A and B10.S) were immunized with the antigen and were tested for the ability to produce both IgE and IgM antibodies. B10.S(9R) raised significant IgM antibody as well as B10.A and B10.S. However, B10.S(9R) produced IgE antibody poorly to *T. vaginalis*. Thus the Ir-*Tr. v.* is located in the left-hand side of the I region from the I-B subregion to the K region. Finally, other strains, A, A.TL and A.TH mice were immunized with the antigen in order to examine the influence of K region on the magnitude of IgE antibody response to the antigen. A and A.TL mice gave good IgE and IgM responses to *T. vaginalis* antigen. On the other hand, A.TH mice elicited intermediate IgE antibody response (1:128.0) (A.TH v.s. A.TL; $P<0.05$) although the same mice could produce high titer of IgM antibody. These data clearly indicate that the K region does not influence the IgE antibody responsiveness, and that Ir-*Tr. v.* is located in the I-A and/or I-B subregion of the H-2.

Non-H-2 Linked Ir-Tr. v. *Regulating IgE Antibody Response to* T. vaginalis

We found that the IgE antibody response to *T. vaginalis* is regulated not only by an H-2 linked Ir gene but also by non-H-2 linked Ir gene(s) (Table 1 and Table 2). We observed the significant difference of the IgE antibody responsiveness between B10.A and A/J strains ($P<0.01$). A/J strain raised high titer of IgE antibody to *T. vaginalis* antigen, while B10.A strain, which possess the same $H\text{-}2^a$ as A/J, produced intermediate level of IgE antibody. In addition, A.TH raised higher IgE antibody

Table 2 The non-H-2 controls IgE antibody response to *Trichomonas vaginalis* antigen

Strain	No. of animals	PCA titer[1]		HA titer[1]	
		mean[2]	range	mean[2]	range
Exp I					
BALB/c	6	576.0	(256–1,024)	576.0	(256–1,024)
B10.D2	6	9.0	(4– 32)	181.0	(64– 512)
Exp II					
A/J	6	456.1	(256–1,024)	837.5	(512–1,024)
B10.A	7	156.5	(64– 512)	464.6	(256– 512)

1, 2: see foot note in Table 1.

Table 3 Ig heavy chain allotype linked locus does not influence the IgE antibody response to *Trichomonas vaginalis*

Strain	No. of animals	H-2 haplotype	IgCH	PCA titer[1]		HA titer[1]	
				mean[2]	range	mean[2]	range
BALB/c	8	d	a	401.7	(128–1,024)	560.3	(256–2,048)
B10.D2	8	d	b	31.6	(4– 64)	232.3	(64– 512)
CB-20	6	d	b	407.3	(128–1,024)	415.9	(256–1,024)
BAB-14	8	d	b	564.2	(256–1,024)	477.7	(256–1,024)
C57BL/6	8	b	b	95.0	(32– 256)	227.5	(128– 512)
BC-8	10	b	a	128.0	(64– 256)	282.1	(128– 512)
BALB.B	10	b	a	104.0	(32– 256)	256.0	(128– 512)

1, 2: see foot note in Table 1.

than B10.S and B10.S(9R) mice. The data showing the different responsiveness of these A.TH, B10.S and B10.S(9R) indicate that the influence of non-H-2 on the IgE antibody response exists. Also as shown in Table 2, BALB/c mice which have an H-2^d haplotype produced the high titer of IgE antibody. On the contrary, B10.D2 mice which have the same H-2^d haplotype as BALB/c failed to raise significant IgE antibody (Table 3), while they produced significant IgM antibody, though somewhat less than the BALB/c strain, to the *T. vaginalis* antigen.

We further examined influences of the non-H-2 on the IgE antibody response to the antigen. One of the possible gene to be mapped as Ir-*Tr. v.* is IgCH gene in which various Ir genes have been identified to be located. Various immunoglobulin allotype congenic mice were immunized with *T. vaginalis* antigen as described in the materials and methods. As shown in Table 3, BALB/c mice raised the high titer of IgE antibody to the antigen (1:401.7). On the other hand, B10.D2, which have the same H-2^d poorly responded to the antigen in PCA titer (1:31.6). Ig heavy chain allotype congenic mice, CB-20 and BAB-14 strains gave high IgE antibody responses (1:407.3 in CB-20, 1:504.2 in BAB-14) as well as BALB/c strain. Both CB-20 and BAB-14 strains possess the same background genes of BALB/c except Ig heavy chain linked genes derived from C57BL/Ka. Thus, intermediate responder strain C57BL/6 (C57BL/Ka) derived Ig heavy chain linked genes do not influence the magnitude of IgE antibody response to *T. vaginalis*. Another Ig heavy chain allotype congenic strain, BC-8, which possess the same background genes of C57BL/6 except BALB/c

(high responder) derived Ig heavy chain genes, was examined. No significant difference was observed in anti-*T. vaginalis* IgE antibody responses between BC-8 and C57BL/6 strains. Thus, Ig heavy chain allotype linked genes do not have any influence on the IgE antibody responsiveness. Furthermore, BALB.B mice having H-2^b haplotype derived from C57BL/6 on BALB/c background were examined. The IgE antibody responses of BALB.B mice were apparently lower than those of BALB/c ($P<0.01$) (though somewhat higher than those C56BL/6 (not significant)). These data show that, in the case of H-2^b, BALB/c (high responder) background gene can not influence on the I region-linked Ir-*Tr. v.* control of the anti-*T. vaginalis* IgE antibody response. These points concerning the difference in influence of the non-H-2 on the response between A/J vs. B10.A, BALB/c vs. B10.D2 and BALB.B vs. C57BL/6 will be discussed later.

Discussion

In an attempt to localize genetically the Ir genes regulating the magnitude of anti-*T. vaginalis* IgE antibody response, various H-2 congenic mice, intra-H-2 recombinant mice and immunoglobulin allotype congenic mice were examined. The data presented in this paper indicate that at least two Ir genes are involved in control of the anti-*T. vaginalis* IgE antibody response. The data obtained from the experiments using C57BL/10 background H-2 congenic mice indicate that one is localized in the K region to the I-B subregion of the H-2. The data from the experiments using a background H-2 congenic mice show that the K region of the H-2 does not influence the magnitude of the anti-Trichomonas IgE antibody. Thus, H-2-linked Ir-*Tr. v.* can be mapped into the I-A and/or I-B subregion of the H-2. Non-H-2-linked immune response gene(s) regulating the anti-*Tr. v.* IgE antibody responsiveness can not be mapped in this study. IgCH-linked gene was considered as an applicant gene because Ir gene regulating the antibody responses to alloantigens have been mapped to the locus (Mckenzie, 1975; Taylor *et al.*, 1975). Although immunoglobulin heavy chain allotype congenic strains were utilized to examine this possibility, IgCH-linked gene does not influence the antibody responsiveness.

In the genetic analyses of the influence of non-H-2 genes on the IgE antibody responsiveness to *T. vaginalis*, we observed complex phenomena. The comparison of the IgE antibody responsiveness between BALB/c v.s. B10.D2 and A/J v.s. B10.A clearly shows that non-H-2 gene(s) dominantly influences the anti-*T. vaginalis* IgE antibody response. However, the influence of the non-H-2 gene on the response was hardly observed in another H-2 congenic strain, since BALB.B mice was as same as C57BL/6 in the IgE antibody responsiveness. The non-H-2 background genes of BALB.B strain from high responder BALB/c could not influence the IgE antibody responsiveness. This fact clearly shows that Ir-*Tr. v.* linked I region of the H-2 in C57BL/6 (H-2^b) mice dominantly expresses its genetic regulation of the IgE antibody responsiveness to the antigen. One of the possible interpretation to the inconsistency in genetic control of the IgE antibody response may be attributed to the different situation in H-2 origin. The H-2 of B10.D2 originated from DBA/2 (Shreffler and David, 1975), while the H-2 of BALB.B came from C57BL/Ka (Shreffler, 1979). DBA/2 mice poorly produced the IgE antibody to the antigen (1:20.3 in PCA, 1:241.3 in HA). Although no serological difference of the H-2 between BALB/c and DBA/2 has been reported, differences in these strains may exist at least in Ir gene functions. Another interpretation is that either B10.D2, B10.A or BALB.B H-2 congenic

strains might not be real H-2 congenic strains. If we missed recombinations of some genes (eg. Ir-*Tr. v.*) accompanied with H-2 complex in either B10.D2, B10.A or BALB.B strains, the reason for the inconsistency of non-H-2 background gene effect on the IgE responsiveness between B10.D2 vs. BALB/c, B10.A vs. A/J and BALB.B vs. C57BL/6 might be easily explainable. So far, we do not have any definitive answer for these possibilities which remain to be analysed.

The HA antibody response to *T. vaginalis*, dominantly of the IgM class, seems not to be regulated by the Ir-*Tr. v.* gene. Mitchell *et al.* (1972) reported that the IgM response to the synthetic copolymer, (T, G)-A--L which can function both as T independent and T dependent immunogen, is not under Ir gene control, whereas Ir gene control of the production of T dependent immunoglobulin classes, such as IgG and IgE, has also been well documented (Dorf *et al.*, 1975). Thus, it may be possible to interpret that Ir-*Tr. v.* gene does not influence the HA antibody response to *T. vaginalis* antigen because of the IgM class response.

Summary

The soluble extract from *Trichomonas vaginalis* can elicit the IgE antibody in mice. The IgE antibody response to *Trichomonas vaginalis* antigen is regulated and influenced by at least two genes; one localized in the I region (I-A and/or I-B subregion) of the H-2 complex on chromosome 17, the other not linked to the H-2 complex and yet identified. We examined the linkage relationship between the non-H-2 linked gene(s) regulating the magnitude of anti-*Trichomonas* IgE antibody response and the immunoglobulin (Ig) heavy chain genes by using various Ig heavy chain allotype congenic mice. The data indicate that the non-H-2 linked gene(s) controlling the magnitude of the IgE antibody production is not linked to Ig heavy chain gene.

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腟トリコモナスに対する免疫応答 II.
マウスにおける抗腟トリコモナス IgE 抗体応答を統御する免疫応答遺伝子の遺伝的連関について

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腟トリコモナス由来可溶性物質によりマウスにおいて IgE 抗体産生を惹起することができる．この腟トリコモナス抗原に対する IgE 抗体応答は少なくとも２つの遺伝子によって統御されている．その１つは，第17染色体上の H-2 complex の *I* 領域 (*I-A* および *I-B* 亜領域ないしそのいずれか) に存在するものであり，他の１つは H-2 に連関していないものである．そこで，後者について，免疫グロブリンH鎖アロタイプに関し congenic なマウスを用い，免疫グロブリンH鎖遺伝子との連関の有無について検討した．その結果，抗腟トリコモナス Ig 抗体の応答性の程度を統御する H-2 非連関免疫応答遺伝子は，免疫グロブリンH鎖遺伝子に連関していないことが示された．